IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2663
Steven M. RUBEN	Art Unit: 1644
Appl. No.: 10/662,429	Examiner: HUYNH, PHUONG N.
Filed: September 16, 2003	Atty. Docket: 1488.1890003/EJH/SAC
For: **Apoptosis Inducing Molecule I**	

Supplementary Declaration of Ann Ferrie
Ruben Exhibit #145

Paper No. _____

Filed on Behalf of Party Ruben:

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES
(Administrative Patent Judge Sally Gardner Lane)

STEVEN M. RUBEN

Junior Party,
(Application No. 08/816,981),

v.

STEVEN R. WILEY
and RAYMOND G. GOODWIN

Senior Party,
(Patent No. 5,763,223).

Patent Interference No. 105,077

SUPPLEMENTARY DECLARATION OF ANN FERRIE

Ruben EXHIBIT 2145
Ruben v. Wiley et al.
Interference No. 105,077
RX 2145

SUPPLEMENTARY DECLARATION OF ANN FERRIE

I, ANN FERRIE, declare and state as follows:

1. I joined Human Genome Sciences, Inc. (HGS) in March 1993 as a Research Associate under the direct supervision of Steven M. Ruben, who at that time was a Scientist in the Department of Molecular Biology. At that time I was known as Ann Kim. I worked as a Research Associate under Dr. Ruben's supervision until I left HGS in June 1998. I have been asked by patent counsel for HGS to supplement my Declaration of June 24, 2004 (RE86). More specifically, I have been asked to describe one of the experiments I carried out at HGS in connection with the development of the protein known as Apoptosis Inducing Molecule I ("AIM-I"). This experiment was recorded at pages 33-34 of my notebook number 202 (RE90)

2. On August 25, 1994, I performed an *in vitro* transcription-translation experiment using plasmid HTPAN08S04 as template DNA and recorded in concurrently in my notebook number 202 (RE90), page 33) to produce the protein encoded by the AIM-I sequence. This experiment was carried out using a commercially-available rabbit reticulocyte lysate system ("TNT"). The HTPAN08S04 plasmid includes a promoter recognized by the RNA polymerase of bacteriophage T3 ("Pol T3") that is positioned upstream of the AIM-I coding sequence. An aliquot of HTPAN08S04 plasmid DNA was combined with rabbit reticulocyte lysate, Pol T3, a "methionine-free" mixture of amino acids, ^{35}S-labeled methionine, a ribonuclease inhibitor ("RNasin"), and buffer. This reaction mixture was incubated for a time sufficient to allow transcription of the AIM-I coding sequence to provide messenger RNA (mRNA) that could then

be translated by the rabbit reticulocyte lysate into protein labeled with ^{35}S-methionine. I took a 5 µL aliquot of the reaction, added 20 µL of protein sample loading buffer (which contains detergent (SDS), 2-mercaptoethanol, bromophenol blue, buffer (Tris), EDTA and water) to that 5 µL aliquot, and then heated the mixture at 95°C, thereby terminating the reaction in that sample. I then fractionated the heated sample by gel electrophoresis on an acrylamide gel along with suitable protein standards of known molecular weight. After the electrophoresis was over, I fixed the gel with a mixture of methanol and acetic acid. I then amplified and dried the gel after which I exposed it to X-ray film overnight. The developed autoradiogram (page 34 of RE90) revealed the presence of a radioactive protein at approximately the predicted molecular weight of 30.9 kilodaltons for AIM-I, in the lane identified as "HTPAN08SO4-Fas," which demonstrated that the AIM-I protein had been synthesized *in vitro*.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or any patent issuing thereupon.

July 15, 2004
Date:

Ann M. Ferrie
Ann Ferrie